**[TOMO-POL] Polarization optical diffraction tomography for high-throughput label-free lipid droplets morphological analysis in living cells.**

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The development of biological processes and the structural organization of biochemical compounds largely depend on the ability to visualize them. This need has become the stimulus for global efforts aimed at developing the capabilities of biomedical and clinical facilities to conduct key cell and tissue research. Despite its long history, optical microscopy continues to evolve, introducing groundbreaking imaging techniques that offer nanometer-level resolution, deeper imaging, faster acquisition, and richer informational content.

One important microscopic imaging method is fluorescence microscopy. It uses very small particles that glow when exposed to light of a specific color. This allows scientists to see a specific part of the sample while the rest remains dark. This technique has developed significantly and allows for viewing even smaller things than before. However, it also has its downsides, for example, it can harm the cells being studied or require additional preparatory procedures, and it does not allow for quantitative measurements of the structures being examined.

Therefore, scientists are increasingly using methods that do not require additional staining, such as Quantitative Phase Microscopy (QPM). This technique allows for non-invasive imaging of cells and their structures without affecting their structure or physiology. This technique is extremely gentle and also allows for quantitative analysis of the parameters of the samples being examined.

The proposed project aims to create a new system that will allow for even better analysis of cells, with particular emphasis on the application for examining lipid droplets. The developed method, Polarization Optical Diffraction Tomography (P-ODT), will use polarization and lighting from multiple directions for three-dimensional analysis of the parameters of the cells being examined. Lipids stored in lipid droplets (LDs) are used as an energy reserve, a building block of organelles, and for the synthesis of steroid hormones. We will try to answer questions about how the phase transitions of the liquid-crystalline form of cholesterol esters, which are structural elements, affect the LDs: is it of physiological significance? Is it related to stress, pathology, or physiological processes? Does it limit the accessibility of lipids to proteins? Current studies of LDs mainly use laborious, resource-intensive, and expensive electron microscopy. Birefringence can be detected using qualitative polarization microscopy, but this technique cannot be used to quantitatively determine the parameters of LDs. Hence, Polarization Optical Diffraction Tomography (P-ODT) is the ideal tool for analyzing the parameters of LDs, as it has already been shown that when LDs absorb cholesterol esters, their structure changes from amorphous to liquid crystalline, which affects their refractive index and birefringence.

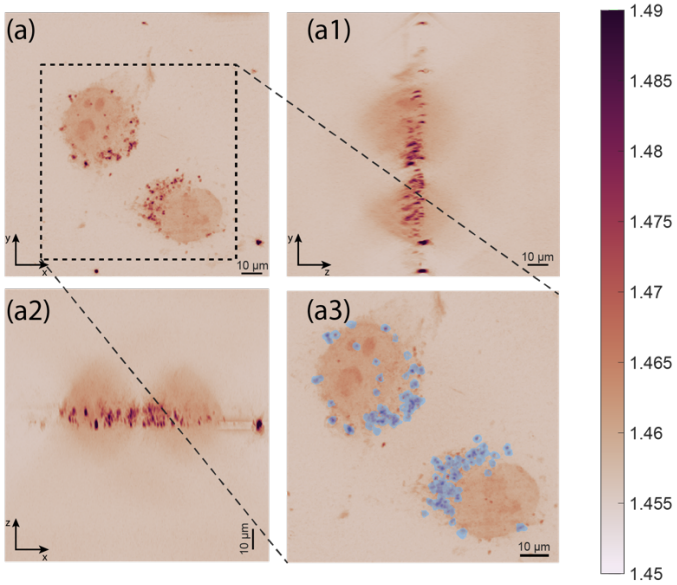


*Fig. 1. Preliminary results of imaging cells with lipid droplets using optical diffraction tomography system. The minimal intensity projection of HeLa cells with prominently marked lipid droplets, the colormap represents the values of the refractive index.*